the claims into better condition for allowance. Support for the amendment to each of claims 67, 75 and 77 is found on page 8, lines 11-13, of the specification. Dependent claims 73 and 83 have each been amended to delete "substantially free of EPA", as the phrase is no longer necessary in view of the amendment to independent claims 67 and 77. Support for the amendments to claims 76, 86, 87 and 94 can be found on page 9, first paragraph, and in example 4 and accompanying Table 2, which provide for the preparation of a microbial oil blend which contains no EPA. The amendment to claim 80 simply puts the claim in independent form.

The examiner rejected claims 67-92 and 94 under 35 U.S.C. § 112, first paragraph, on the basis that the specification did not support the limitations that the fatty acids be in triglyceride form and be substantially free of EPA. The latter rejection has been obviated by the amendments set forth above to the claims. With regard to the examiner's concerns regarding triglycerides, Applicant respectfully submits that this is not new matter. Applicant notes that the present application specifically incorporates therein by reference the specifications of two commonly owned patent applications which describe in detail methods for obtaining microbial oils enriched in either DHA or ARA. The DHA application specifically states that the DHA produced comprises at least 90% triglyceride (see page 10, lines 34 to page 11, line 7). The ARA application specifically provides that the ARA produced comprises either a triglyceride or a combination of a triglyceride and phospholipid (see page 15,

lines 20-24 and page 17, lines 31-34). Thus, this description of a characteristic of the products was part of the specification of the present application as filed and addition of the limitation to the claims does not constitute adding new matter to the application.

All of the claims pending in this application have been rejected under 25 U.S.C. § 103 as obvious in view of the teachings of Japanese Patent Application 196,255 (the Suntory application) and PCT Application WO89/00606 (the Long application) in view of the teachings of Clandinin et al. and Traitler et al. The examiner stated that Suntory discloses adding microbial oils containing polyunsaturated fatty acids (PUFAs), such as ARA, EPA and GLA, to infant formula. The Long application was cited as disclosing supplementing infant formula with microbial oils comprising DHA and EPA. The examiner stated that the claimed subject matter differs from the primary references in claiming the addition of GLA from plant and fish oils and that to so supplement infant formula would have been obvious from the teachings of Clandinin et al. and Traitler et al. Clandinin et al. were cited for teaching the addition of fatty acids from fish oil to infant formula, and Traitler et al. were cited as teaching the addition of GLA-containing black currant oil to infant formula. The examiner asserted that the choice of various ratios for the components constituted only optimization of the composition which is within the skill of the artisan. This rejection is traversed.

The claims of the application, as amended, are directed to blends of oils comprising certain long chain polyunsaturated fatty acids and to the use of these blends to supplement infant formula to provide a formula that contains these PUFAs in amounts comparable to the amounts found in human breast milk. The prior art references relied upon by the examiner, taken singularly or in combination, do not enable one to obtain an infant formula having this highly desirable characteristic.

Specifically, the present application teaches and claims supplementing infant formula with ARA- and DHA-enriched microbial oils to provide a product in which the amounts of ARA and DHA are comparable to the amounts provided in human breast milk. In addition, the resultant formula either is free of a third PUFA, EPA, or contains EPA in a small amount that is comparable to the amount of EPA present in human breast milk.

As Applicant has explained in a response to a previous Office Action, EPA is a desirable fatty acid component of certain products. Over the last several years, however, there has been increasing evidence accumulating that relatively high amounts of EPA are not desirable components of other products, particularly infant formula. Studies have shown that high levels of EPA in infant formula can be deleterious to infant development. Applicant previously cited to the examiner Carlson, S.E., et al., *Essential Fatty Acids and Eicosenoids*, page 192, A. Sinclair and R. Gibson, eds. (1992), who recommended that infant formula be supplemented with DHA alone, rather than a combination of DHA and

EPA, in view of their findings that the administration of formula comprising significant amounts of EPA resulted in negative effects on infant growth and psychomotor development.

The fatty acid composition of human milk is shown in Table 6 (page 136) of *Omega-3 Fatty Acids in Health and Disease*, Robert Lees and Marcus Karel, eds., (1990). A copy of this table also has been provided previously to the examiner. As shown therein, EPA generally comprises only about 0.03% of the fatty acid composition. ARA, in contrast, comprises about 0.59%, and DHA comprises about 0.19%, of the fatty acid composition of human breast milk[1]. Manufacturers of commercial infant formula have tried to duplicate the fatty acid composition of breast milk, but have been unable to do so. Consequently, no commercially available formulas to date contain DHA and ARA in the desired amounts without also providing EPA in amounts which exceed the very small amount present in human breast milk. Prior to Applicant's invention, there were no sources for DHA and ARA that would provide commercially significant quantities of these desirable fatty acids without the concomitant production of significant amounts of EPA. In addition, microbial oils of the Applicant's invention provide DHA and ARA in triglyceride form. As this is the form in which these PUFAs are provided in human breast milk, Applicant is able to produce oil blends which can be

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[1] These percentages represent average levels. In individual instances, the amounts of one or more of these fatty acids may vary. See, for example, Table 1 of the Clandinin et al. reference cited by the examiner.

added to infant formulas to provide a final product in which the long chain PUFAs of interest are present in both the same form and the same amounts as they are found in human breast milk. As a result of Applicant's work, one now can very precisely obtain, in an economically viable process, a composition comprising desired absolute and relative amounts of DHA and ARA in triglyceride form that are free of undesired amounts of another long chain PUFA, EPA.

Similarly, claims 88-91 set forth above are directed to compositions providing a combination of a microbially-produced oil comprising DHA and an oil comprising GLA. As discussed in the specification, GLA is a precursor of ARA and, in certain circumstances, it may be desirable to provide the precursor, rather than ARA itself, in a composition. Again, a distinguishing feature of the claimed compositions is that it provides the two fatty acids of interest in an environment that is free of unwanted EPA.

The references cited by the examiner do not teach or suggest the oil blends or supplemented infant formulas of the present invention. The claims of the application are limited to microbial oil blends comprising specific ratios of ARA and DHA and no more than a low amount of EPA. More specifically, the claims are directed to such blends which comprise ARA and DHA in a ratio which can range from about 5 parts ARA and 1 part DHA to about 20 parts ARA and 10 parts DHA and which is either free of EPA or can comprise 1 part of EPA. Although the two primary

references were cited by the examiner as teaching the components of Applicant's oil mixtures, neither of these references discloses or suggests obtaining or using oils having the key characteristics of the oil components of the Applicant's invention. In each of the two primary references it is deemed acceptable, and even desirable, to supplement infant formula with lipids containing significant amounts of EPA, and neither reference discloses the use of oils enriched in DHA and ARA but deficient in EPA as a nutritional supplement. The deficiencies of the primary references are not compensated by the secondary references cited in the Office Action.

The Suntory application is directed to the addition of one or more fatty acids to infant formula. The application teaches the addition of the fatty acids gamma linolenic acid (GLA), bis-homo-gamma-linolenic acid (DGLA), arachidonic acid (ARA), eicosadienoic acid (EDA) and eicosapentaenoic acid (EPA), either alone or in combination, to manufactured milk. The application makes no reference to DHA.

Suntory discloses that the fatty acids can be in the form of free fatty acid, fatty acid esters, oils and fats, hydrolysates of the oils and fats, or esterified products of dissolved matter of the fats and oils. The Suntory application provides that these additives can be produced by yeast methods or fermentation methods, but the application provides very little information as to sources of the fatty acids. Although the application contains examples (see examples 1 and 2) in which a single fatty acid is

added to milk, there is no discussion as to the source used to produce the fatty acid, how it was obtained to the exclusion of, or isolated from, other fatty acids, or how to obtain it in the form of a triglyceride. The only microorganisms mentioned in the application are *Mortierella* organisms, but in the examples in which a *Mortierella* strain was cultivated to produce fatty acid, the product obtained was a mixture of fatty acids that contained ARA and significant amounts of EPA, as well as other PUFAs. For instance, in example 4 of the application, *Mortierella aeromonas* was cultured to produce an "oil and fat" that was extracted from the organism and converted to produce an ethyl ester product. The composition of the product was found to contain the ethyl esters of palmitic acid, stearic acid, oleic acid, linolenic acid, GLA, DGLA, EDA, ARA and EPA. The example states that the amount of EPA present in the mixture is 50% of the amount of ARA. This composition containing the mixture of ethyl esters was added to powdered milk. Clearly, this is distinguishable from the Applicant's invention, in which the ratio of ARA:EPA in the oil blend is at least 5:1 and the blend comprises no more than one fifth as much as ARA.

Example 6 of the Suntory application teaches adding the ethyl esters of GLA, EDA, DGLA, ARA and EPA to cyclodextrin in relative weight percentages of 2:1:6:4:8 and adding the resultant product to powdered and liquid milk. In this example, in which specific relative amounts of each of the fatty acids of interest are provided, *twice as much* EPA as ARA is added to milk. The use

of such mixtures to supplement infant formula is fundamentally different from the invention claimed by the Applicant.

In addition, although the Suntory application provides that the fatty acids or their salts or esters can be utilized without further modification, it teaches that it is a "good idea" to first encapsulate the substance in cyclodextrin. In examples 1 and 2 of the application, the applicants teach adding 1 gram of cyclodextrin powder containing 5 % of one of the fatty acids of interest to 1 kg of a powdered milk. This is the equivalent of adding 50 mg of fatty acid to 1 kg of milk powder. If the fatty acid is ARA (example 2), this amount would be much less than the amount necessary to match the amount found in human breast milk. Typically, there are 250 g fat per kg of milk powder (see, for example, Similac™, Ross Laboratories). Human breast milk comprises about 0.5-0.7 % ARA, as cited above. Thus, to provide a milk powder having an amount of ARA comparable to that in human breast milk, one would need to add about 1.3-1.8 g ARA per kg of the powdered milk. Suntory's example thus teaches only adding about three one-hundredths (3%) of the desired amount of ARA. In order to provide a milk product having an amount of ARA comparable to the amount found in human breast milk, approximately 30-40 g of the Suntory ARA-containing cyclodextrin would have to be added to 1 kg of powdered milk. Such an amount of an artificial additive like cyclodextrin certainly would not be desirable in infant formula, where the goal has always been to

provide a formula as close to natural, human breast milk as possible.

Thus, in contrast to the Applicant's invention, the Suntory application does not teach or suggest how to obtain an infant formula comprising ARA and EPA in amounts comparable to the amount of ARA found in human breast milk.

The second reference cited by the examiner, the PCT application by Long, discusses culturing a variety of microorganisms to produce omega-3 fatty acids (such as DHA and EPA) that then can be used in food, cosmetic and pharmaceutical products. This application contains only prophetic examples. In each of examples 1-5, Long provides general procedures for culturing any of a variety of microorganisms, including fungi and microalgae, and then harvesting the cells and recovering the lipids produced. At the end of the examples, Long states that the lipid fractions contain omega-3 fatty acids, which then are esterified to produce the corresponding methyl esters. He goes on to provide that the omega-3 fatty acids

> may constitute as much as 10 to 50% of the total fatty acid fraction. They are generally contained in phospholipids, glycolipids, mono-, di-, or triglycerides, *and* sulpholipids, or as the free acids, but are not limited to these forms.

(page 8, lines 7-10 [Emphasis added]).

Long does not teach or suggest obtaining an oil enriched in a single omega-3 fatty acid, such as DHA, much less obtaining such an oil without the concomitant production of significant

amounts of EPA, another omega-3 fatty acid. Applicants are claiming oil compositions in which one component is an oil enriched in DHA, and a key feature of the oil composition is that it is substantially free of EPA. Long teaches only how to obtain a variety of omega-3 fatty acids in a complex and unseparated form. Anyone reading the passage quoted above from the Long application would believe that one could not obtain a single omega-3 fatty acid, such as DHA, by cultivating a microorganism.

Additionally, as also is apparent from the Long passage quoted above, Long provides that from 10-50% of the total fatty acid fraction produced may constitute omega-3 fatty acids. The total amount of DHA, one particular such fatty acid, in one particular form of lipid (triglyceride), therefore is likely to be quite small. Thus, from Long's disclosure, one knows only that any of a list of eukaryotic microorganisms can be cultivated and will be found to contain a variety of omega-3 fatty acids and that these fatty acids can be present in a complex, unrecoverable form. There is absolutely no teaching in the Long application that an oil enriched in a single desired fatty acid, DHA, can be obtained from a microbial source, recovered and then used with an oil enriched in ARA or GLA to provide a nutritional supplement, such as a supplement for infant formula.

In Applicant's response to the previous Office Action, Applicant showed that the procedures set forth in the Long application do not teach one how to obtain a DHA-enriched oil. Applicant faithfully and precisely carried out the specific

prophetic examples in the Long application, culturing a number of different strains of *C. cohnii* using culture media comprising various concentrations of carbon and nitrogen in accordance with Long's teachings. None of these experiments resulted in the production of a single cell oil enriched in DHA. The results of these experiments were provided to the examiner in a Declaration Pursuant to 37 C.F.R. § 1.132. As can be seen from the declaration, time after time, the culturing produced zero to very low yields of DHA. At no time did the Applicant obtain a recoverable oil comprising a significant amount of DHA and certainly did not obtain an oil comprising at least 25% DHA, as specified in a number of the dependent claims of the application.

The secondary references do not cure the deficiencies of the primary references. Clandinin et al. teach the use of egg yolk lipid and fish oil to provide an edible fat composition for incorporation into an infant formula. Nowhere do Clandinin et al. recognize, teach or suggest that microbial oils can provide the fatty acids useful for supplementing nutritional products such as infant formula, much less that such oils can be used to provide desired amounts of DHA and ARA without also providing excessive amounts of EPA.

Clandinin uses fish oil as a source of the omega-3 fatty acids to be added to infant formula. Fish oil can comprise DHA, but it also generally comprises significant amounts of EPA. If fish oil is added to an infant formula in a sufficient amount to provide an amount of DHA comparable to that found in human breast

milk, the level of EPA in the formula typically will be much higher than the level of EPA in breast milk. Table 7 of the Clandinin patent provides the fatty acid composition of infant formula made with fish oil. The amount of ARA is only about one quarter of the amount found in human breast milk (0.13% rather than 0.6%), yet the amount of EPA is more than 60 times higher than the amount in breast milk (2.03% vs. 0.03%)[2]. Clandinin's formula also has no detectable amounts of DHA. As has been noted above, recent scientific evidence indicates that the administration of high levels of EPA to infants can be deleterious to their development. Specifically, for example, it has been shown that fish oil generally is not suitable for infant formula supplementation as the EPA inhibits the conversion of linolenic acid to ARA in the infant's body. See Carlson, S.E. et al., referenced above, and Bjerve, K.S., et al., *Am. J. Clin. Nutri.* 57(supp): 801S (1993), a copy of which was provided with Applicant's response to the previous Office Action. Thus, although fish oil provides one desirable fatty acid, DHA, it can hinder infant structural and organ development by limiting availability of a second important fatty acid, ARA.

Clandinin also discloses the use of egg yolk lipid as a source for omega-6 fatty acids. Approximately 2% of the total lipid in egg yolk lipid is ARA; little EPA or DHA is found in egg

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[2] Even if one uses Clandinin's own calculations for the fatty acid composition of breast milk, the amount of EPA in the fish oil-containing infant formula of Table 7 still is about 17 times higher than the amount of EPA in breast milk (2.03% vs. 0.12%).

yolk lipid. The long chain fatty acids in egg yolk lipid, however, are in the form of phospholipids. In contrast to this, Applicant provides oils in which the desired fatty acids are in the form of triglycerides, the same form as they are found in human breast milk. Clandinin does not teach or suggest how to achieve a composition which provides key fatty acids in the same form and in the same amounts as found in human breast milk and which does not contain amounts of a fatty acid (EPA) now believed to be deleterious to infant development greater than the trace amounts that exist in human breast milk.

The last reference cited by the examiner, Traitler et al., discloses only obtaining an oil comprising GLA from a fruit. This reference does not teach or suggest combining such oils with microbial oils enriched in DHA and either free of or containing only low levels of EPA.

To summarize, none of the cited references, when considered independently or in combination, disclose or suggest mixtures of oils enriched in ARA and DHA, wherein the mixture is further characterized by, at most, low levels (no more than 20% of the amount of ARA) of EPA. In addition, the cited references do not teach or suggest mixtures of oils comprising a microbial oil enriched in DHA and an oil comprising GLA, wherein a further characteristic of the mixture is that it does not comprise significant amounts of EPA. The cited references further do not teach how to obtain an infant formula comprising DHA, ARA and EPA

in the same form as, and in amounts comparable to the amounts of, these fatty acids in human breast milk.

In view of the foregoing amendments and discussion, Applicant respectfully submits that the claims of the application are in condition for allowance.

Respectfully submitted,

ROTHWELL, FIGG, ERNST & KURZ, p.c.

By _____

E. Anthony Figg
Attorney for Applicants
Registration No. 24,195

555 13th St., N.W., Suite 701-East
Washington, D.C. 20004
Telephone: (202)783-6040